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THE SPACE-TIME PATTERN OF SEGMENT FORMATION IN ARTEMIA SALINA

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INTRODUCTION

The present work was carried out in an attempt to arrive at a primary understanding of the regularities and laws in the phenomenon of metameric segmentation, as related to the shape and size of animals. To date this phenomenon, although of widespread occurrence amongst the higher animal phyla and thus probably an integral part in the more complex patterns of evolutionary organization, was nevertheless surprisingly rarely, if at all, subjected to analytical inquiry. The reason for this can probably be found in an essential lack in the past of well-defined concepts about the interrelations between mass, shape, growth, and degree of development of living organisms. The problem of segment formation in relation to size and shape is primarily one involving a clear appreciation of the dynamic geometry of living matter, and initial insight into the problem can therefore only emerge from rigorous observation on a quantitative level, followed preferably by geometrical and mathematical analysis. Such a method has been employed in the present work, and the results gained are conclusive enough not only to point the way for further study of the problem at hand, but also to promise reasonable success in the application of the quantitative, geometrical method to questions of biological space-time pattern in general.

The choice of *Artemia* has proven particularly fortunate for a study of metameric segmentation. The animal, held to be amongst the most primitive of living Crustaceans (Lockhead, 1941), develops few, if any, specialized structural features which would ordinarily tend to obscure the fundamental processes of morphogenesis. Moreover, the development of as many as nineteen body segments, a further primitive trait, is of obvious advantage in the investigation of the underlying principles of formation. Also, *Artemia* is easily obtained and can be reared in the laboratory without difficulty.

METHODS AND MATERIALS

Larvae of *Artemia salina* were obtained from commercial, air-dried egg cysts. Since excystment is retarded or inhibited in water above a certain salinity (Jennings and Whitaker, 1941), water of a specific gravity of 1.020 was used throughout as the initial medium. The egg shells cracked open usually 12 to 18 hours after contact with the water, and emergence of the larvae (Whitaker, 1940) took place

between 18 and 24 hours. Portions of five stock solutions of brines with different salt concentration were employed as further media. The solutions were obtained from the original sea water by either diluting with doubly glass distilled water or concentrating with NaCl to specific gravities of 1.022, 1.033, 1.047, 1.066, and 1.085, respectively. All solutions were vigorously aerated daily, and possible deviations from the proper specific gravity were adjusted in weekly intervals. All work was carried out at room temperature, corresponding to an average water temperature of 21–22° C. As soon as the embryos had emerged, still enclosed within the fine hatching membrane, they were transferred to water from either of the five stock solutions. The moment of hatching, occurring within 24 to 30 hours after the cysts had first made water contact, was taken as zero time for all further determinations.

For observation the larvae were reared singly, in heavy crystal watch glasses. In the course of several observational series, a total of up to 100 individuals were observed, at least for certain periods of their development; of the 100, about 25 individuals, evenly distributed among the solutions, were reared from hatching to the adult stage. The presence or absence of a molted shell, the time, the temperature, the stage of development reached, and a series of measurements on bodily proportion were recorded for each individual twice daily in the earlier stages and daily for later stages. The animals were fed once every two days on a sea-water-yeast suspension. Each watch glass containing an animal was covered so that evaporation was nearly abolished, but a minimum of air circulation was always allowed for to equilibrate the CO₂ released by the yeast and the animal. The water was changed at two-day intervals for the younger stages and daily for older ones.

Larval body measurements were taken under the microscope with the help of a hemocytometer slide whose grid allows the direct reading off of lengths of 50 micra and consistent estimations of lengths of 10, 20, and 30 micra. If the larva is placed on a coverslip with a minimum of water, the whole can be adjusted in relation to the grid; evaporation is sufficiently slow to allow five or six measurements at a time. The error inherent in this method, viz., the parallax due to the thickness of the coverslip, is small enough to be negligible; also, since all measurements were taken in this way, the relative values are consistent.

PRELIMINARY OBSERVATIONS

Barigozzi (1939) and Rugh (1941) observed the total developmental time from hatching to the adult stage of *Artemia* to be 3 to 4 weeks. This is true as a broad generalization, but with the egg cysts in the various salinity media here employed, certain statistically preferred tendencies become apparent, expressed empirically by

$$D_x = D_0 \cdot S_x^{-6.8}$$

where D_x is the time, in days, for complete development from hatching in a solution of salt concentration x ; D_0 , the (hypothetical) time, similarly, for development in distilled water (the algebraic value turned out to be 36.55); and S_x , the specific gravity of the solution of concentration x . This relation holds good only in a statistical sense, within a specific gravity range of 1.020 and 1.1; it indicates that with higher salt concentrations the rate of development tends to be greater (Fig. 1). Irrespective of concentration a sigmoid curve of growth is always obtained.

Morphologically, different salinities have no differential effect on relative body proportions, a result to be expected in view of the conclusions of Bond (1932). An inverse relation between total size and salinity, observed by Bond, Heath (1924), and Warren (1938) for larvae from non-excysted eggs in the natural habitat, however, could not be observed for the excysted larvae here used; in the latter, total sizes are identical at equivalent stages of development, irrespective of salinity.

The number of molts between hatching and sexual maturity is not constant. Even when reared in the same medium, slight differences in molting frequency between several larvae may occur. Moreover, there exists a rough statistical relation between salinity and the total number of molts, approximating closely the

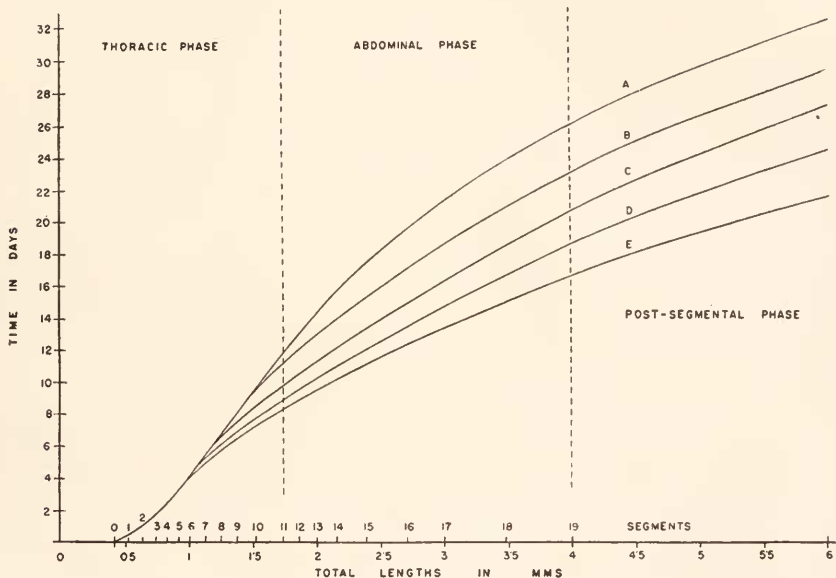


FIGURE 1. The effect of salinity on developmental time, from hatching to sexual maturity; absolute growth curves. Abscissa: total larval length; Ordinate: time in days. A-E, media of salt water, specific gravities from 1.022-1.085 respectively; numbers 1-19 above abscissa refer to number of body segments present.

above relation between salinity and the time required for complete development; in general, however, the number of molts for a given salinity is somewhat lower than the number of days required for development. In larvae from excysted eggs and under artificial food conditions, a range of 12 to 16 molts was observed between hatching and maturity, at a specific gravity of 1.085; this compares with 25 to 29 molts at a specific gravity of 1.022, and gradually decreasing molting frequencies for the intermediate salinity ranges. A staging of larval development according to molts, as Heath has done for non-excysted individuals, would therefore not be possible in the present case. Heath's 13 stages would hold for excysted larvae only when reared in brine of a specific gravity of 1.085; even then certain definite differences in the degree of development of equivalent molting stages can be

observed, as comparison of Heath's descriptions with those below makes apparent.

With increasing developmental age the duration of instars increases; a 12 to 24 hour interval between molts in the younger stages compares with 24 to 30 hour intervals in older ones. The two factors of salinity and developmental age also determine the size increase between molts; for higher salinities, as well as for older larvae, the size increase is greater. There is no observable relation however between the time at which a molt occurs and the size or the developmental stage attained, irrespective of whether test larvae are reared in the same or at different salinities. Molting is also greatly influenced by the food supply. Starving animals do not molt; after 3-5 days an abortive attempt at molting is made which usually results in the death of the animal. Conversely, overfed larvae may molt twice in rapid succession without undue increase in size.

ANALYSIS OF SEGMENT FORMATION

Observations and definitions

The larval development of *Artemia* can best be dealt with in terms of the number of body segments present. The first three segments become visible almost simultaneously at a total larval size of 0.745 mm. (stage 3), after the embryonic yolk has been digested away, and the termination of the hatching, nauplius, and metanauplius stages can therefore be represented as the termination of stages 0, 1, and 2, respectively; at the end of any following stage the stage number will thus indicate directly the number of body segments present. It will be convenient to distinguish between a thoracic period of development, comprising the first 11 stages, and an abdominal period, including stages 12 to 19; the latter can again be divided into a genital period (stages 12 and 13) and a post-abdominal one (stages 14 to 19).

Except for the first three, each individual segment is initially recognizable as a transverse ring of thickened mesoderm, the segment rudiment, immediately underneath the otherwise smooth epidermal layers (segmental stage *a*). Later, partial transverse constrictions appear externally in the epidermis and the chitin, in a plane just posterior to that of the segment rudiment (segmental stage *b*). Eventually, the constrictions become complete and deepen, with a concomitant bulging out of the body wall in the region of the segment rudiment (segmental stage *c*). At this stage, the segment can be considered "laid down," its shape resembling more or less a short cylinder. In thoracic segments, appendage buds appear in stage *c* ventro-laterally, on either side. The segments are considered mature when their pairs of swimming appendages first become independently motile. Stages *a* to *c* of the first and second, and stages *a* and *b* of the third segment can never be clearly seen; the first stages of these segments are attained prior to hatching and during the nauplius and metanauplius phases, when the presence of dense yolk conceals details of structure. As these segments become plainly visible in the third stage of the thoracic period, segment 3 is in stage *c*, but segments 1 and 2 are already correspondingly ahead, both in size and the degree of their development.

At the end of the thoracic period the 11th segment has reached stage *c* and the first five segments have become mature. The 11th segment attains maturity at the end of the abdominal phase of development (stage 19). Appendage buds similar to those on more anterior segments also develop on segments 12 and 13. But instead of developing into swimming appendages the buds on either side of

both segments enlarge, and in the female fuse into a sac in stage 18, forming the left and right brood pouch; no male larvae were investigated. The remaining six segments develop similarly from segment rudiments, but no appendage buds are ever formed and stage *c* represents the first stage of maturity. At the end of the abdominal period the 19th and last segment has become mature. In the head, the ocellus becomes pigmented in stage 2 and the compound eyes in stage 4. The maxillae and maxillulae also form in stage 2. The end of stage 19 marks the time when the gnathobase and the setae have been lost entirely from the second antenna. After stage 19 an arbitrary number of non-segmental stages ensue before sexual maturity is reached.

When individuals in identical stages of development from the same or from different salinity media are compared, it is strikingly apparent that total lengths and body proportions in general fall within well-defined size-classes; the deviations from the underlying averages in no case exceed ± 3 per cent. In Table I the averages of a variety of body measurements are shown, from the 25 individuals watched throughout development, with the stage number as the basis of calculation; these values, within ± 3 per cent, are true for individuals from any of the salinities here examined. A schematic diagram of an *Artemia* larva indicates, in Figure 2, how the various entities have been defined. Head length is understood to include maxillar and maxillular segments. The length of a segment refers to axial and the width to its lateral extent. Total abdominal length is the length of the segmental portion, whether actually cut up into segments or not, plus the length of a terminating anal piece; the segmental portion is the pygidium of annelid forms, and in *Artemia* is readily distinguished from the anal piece, or urosome, by a constriction. During the abdominal period of development, the segmental abdomen contains a genital region composed of segments 12 and 13, as well as a post-abdomen (presumptive segments 14 to 19) with segmented and non-segmented portions.

From observation and from examination of the data in Table I the following facts concerning the formation of segments in relation to larval shape and size are consistently found to occur:

1. Every thoracic segment when newly formed (stage *c*) has a fixed length of 0.03 mm. and a fixed width of 0.144 mm.
2. Every time a new thoracic segment is laid down in stage *c*, preceding segments increase in length and in width.
3. Throughout the thoracic period, the segmental part of the abdomen has a constant average length of 0.249 mm.; its anterior width, being slightly smaller than the width of the newest segment, is also constant ($C = 0.142$ mm.).
4. During the thoracic period, the lateral contour-lines of the thorax are straight lines converging posteriorly; the lateral abdominal contour-lines are also straight, but generally they converge with a greater degree of taper than the thoracic contours.
5. Appendages are longer the more anterior they are; the line joining the tips of the appendages on one side of the body is more or less a straight line.
6. As the 11th segment appears in stage *c*, the 5th segment has matured and the 19th segment has appeared in stage *a*.
7. Between stage *a* and stage *c* of the thoracic segments, 4 stages of larval development intervene; an interval of more than 4 developmental stages is necessary for an abdominal segment to reach stage *c* from stage *a*.

TABLE I
Segmental development and larval body proportions, in millimeters

Stage Number	No. SR	No. S	SM	Tot. L	HL	T	TAL	A	GL	P	SPAL	U	TS	TSL	PS	W.	WTSL	WA	WASL	WU
0	4	0		0.425	0.206	0.030	0.219	0.215				0.070	0.030	0.030		(0.142)	0.144			0.080
1	5	1		0.524	0.209	0.070	0.285	0.238				0.080	0.040	0.030		0.144	0.144			0.080
2	6	2		0.637	0.249	0.122	0.318	0.241				0.093	0.050	0.030		0.146	0.145			0.085
3	7	3		0.745	0.289	0.185	0.334	0.241				0.100	0.058	0.031		0.150	0.136			0.097
4	8	4		0.841	0.313	0.242	0.343	0.244				0.109	0.065	0.028		0.155	0.139			0.105
5	9	5		0.934	0.339	0.311	0.353	0.235				0.116	0.076	0.033		0.162	0.138			0.105
6	10	6	1	1.025	0.343	0.390	0.371	0.237				0.132	0.086	0.029		0.167	0.139			0.108
7	11	7	2	1.125	0.366	0.480	0.369	0.243				0.136	0.097	0.030		0.179	0.143			0.107
8	13	8	3	1.236	0.382	0.600	0.379	0.243				0.146	0.110	0.031		0.191	0.144			0.111
9	15	9	4	1.386	0.400	0.733	0.386	0.240				0.154	0.120	0.033		0.209	0.143			0.118
10	17	10	5	1.540	0.407	0.861	0.400	0.246				0.180	0.127	0.027		0.225	0.150			0.120
11	19	11		1.716	0.429	0.87	0.426	0.246	0.04	0.290		0.200	0.135	0.035		0.245	0.150	0.150	0.145	0.125
12		12		1.855	0.455	0.87	0.53		0.10	0.325		0.225	0.130	0.035		0.25		0.160	0.145	0.13
13		13		2.000	0.460	0.87	0.65		0.12	0.38		0.25	0.135	0.045	0.06	0.25		0.170	0.148	0.13
14		14	6, 14	2.155	0.475	0.93	0.75		0.14	0.45	0.06	0.24	0.145	0.050	0.08	0.35		0.185	0.150	0.13
15		15	7, 15	2.400	0.520	1.05	0.83		0.16	0.58	0.16	0.27	0.150	0.060	0.09	0.33		0.190	0.140	0.14
16		16	8, 16	2.720	0.550	1.20	0.97		0.165	0.73	0.27	0.25	0.175	0.065	0.12	0.33		0.200	0.137	0.14
17		17	9, 17	3.050	0.570	1.35	1.13		0.185		0.48	0.25								
18		18	10, 18						0.19	0.91	0.75	0.25	0.175	0.075	0.15	0.35		0.210	0.139	0.14
19		19	12, 13 11, 19	3.450 4.000	0.600 0.650	1.50 1.70	1.35 1.65		0.23	1.13	1.08	0.27	0.180	0.095	0.18	0.37		0.230	0.144	0.14

Legend: No. SR, number of segment rudiments; No. S, number of segments present; SM, segments matured in this stage; Tot. L, total larval length; HL, head length; T, thoracic length; TAL, total abdominal length; A, length of segmental abdomen; GL, genital length; P, post-abdominal length; SPAL, length of segmented post-abdomen; U, urosomal length; TS, length of first thoracic segment; TSL, length of last thoracic segment; PS, length of a post-abdominal segment; W, width of first thoracic segment; WTSL, width of last thoracic segment WA, width of first abdominal segment; WASL, width of last abdominal segment; WU, anterior urosomal width.

8. Between stage c and maturity in the first 5 segments, 6 stages of development intervene; 8 stages intervene before maturity of segments 6 to 11.

9. The anterior width of the non-segmented portion of the abdomen during the abdominal phase is of a fixed and constant magnitude and identical to the constant anterior width of the abdomen during the thoracic phase; thus at the end of stage

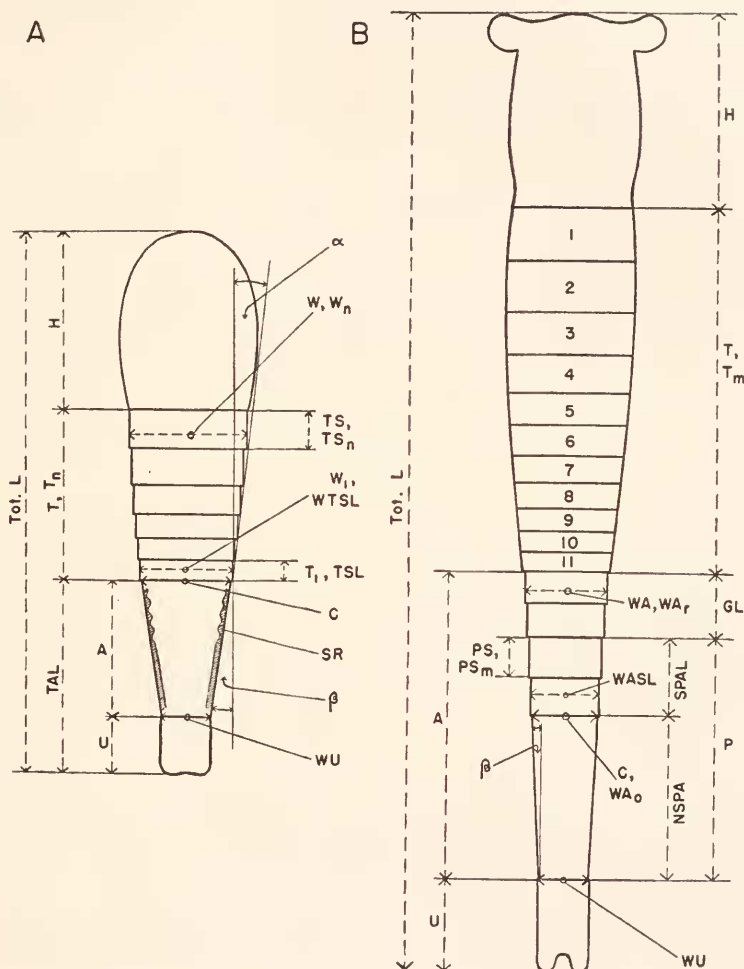


FIGURE 2. Schematic diagram of a larva of *Artemia salina*. A, larva in the thoracic period; B, larva in the post-abdominal period of development. A , length of segmental abdomen (pygidium); α , angle of thoracic taper; β , angle of abdominal taper; C, W_0, WA_0 , constant anterior width of segmental abdomen; C , length of genital region; H , head length; $NSPA$, non-segmented post-abdomen; P , length of post-abdomen; PS, PS_m , length of a post-abdominal segment; $SPAL$, length of segmented post-abdomen; SR , segment rudiment; T, T_n, T_m , length of thorax; TAL , total abdominal length; TS, TS_n , length of first thoracic segment; TSL, T_1 , length of last (newest) thoracic segment; $Tot.L$, total larval length; U , urosomal length; W, W_n , width of first thoracic segment; $WTSL, W_1$, width of last thoracic segment; WA, WA_r , width of first abdominal (12th) segment; $WASL$, width of last (newest) abdominal segment; WU , anterior width of urosome.

19, the width of the urosome is the same as the posterior width of the head in stage 0 when segmental development started. A constant width has seemingly travelled down the larva.

10. At the beginning of stage 12, the segmental abdomen starts to grow in length at a fast rate, having retained a constant length in the thoracic period.

11. Segments 12 and 13 are of equal length at any time after their formation; they are individually always somewhat longer than the 11th thoracic segment and become progressively shorter, relatively, than the 14th segment. At stage 18, 6 developmental stages after segment 12 has reached stage *c*, segment 12 and 13 fuse to form the brood pouch in the female and can then be considered matured. The interval for attainment of maturity is thus equal to the similar interval in the first 5 thoracic segments.

12. Segments 14 to 19 are not of equal length when formed; more posterior segments, when formed, are longer than more anterior ones when formed. Also, any one of these segments has always one-sixth of the length of the post abdomen, and at a given time post-abdominal segments are of equal length.

13. When segment 19 reaches stage *c*, the 11th segment attains maturity.

14. During the abdominal phase, the thorax changes shape in the following way: the 2nd segment becomes longer and wider than the 1st, then the 3rd larger than the 2nd, etc., and the 5th becomes the largest, coincident with the end of stage 19. As a result, the lateral thoracic contours become curved, the widest part of the thorax being at segment 2 in stage 16, at segment 3 in stage 17, etc., and at segment 5 in stage 19.

15. Similar differential increases take place in the appendages; at stage 19, the 5th pair of swimming appendages is longest and appendageal length regularly decreases towards the 1st and the 11th pair. The line joining the appendage tips on one side of the thorax is now also curved.

16. During the abdominal phase, and paralleling the differential increases in the thoracic segments, a progressive dorsal thoracic curvature develops, with an analogous shift backwards of the maximal flexure; the latter arrives similarly at segment 5 at the end of stage 19. Due to this flexure the head now appears bent ventrad.

17. The lateral contours of the abdomen remain straight lines throughout the abdominal phase, with a definite taper directed backwards.

18. At the end of stage 19 segmental development is completed; further development is still to take place in the head. The essential overall shape of the animal as now established, i.e., the possession of a barrel-shaped thorax and a straight tapering abdomen, is carried through to sexual maturity, although changes of detail do still occur.

These observations are now to be interpreted and integrated analytically.

The thoracic phase of development

The lengths of the first thoracic segment, in successive stages, are 0.03, 0.04, 0.05, 0.058, 0.065, 0.076 mm., etc. (Table I). The differences between these values, taken for all 11 thoracic stages, are very close to an average difference of 0.0097 mm. The length of the first thoracic segment in successive stages can

therefore be expressed as an arithmetical series

$$TS_n = (TS_1 - TS_0) + (n - 1) \cdot \Delta s \quad (1)$$

where TS_n refers to the length of the first thoracic segment at stage n ; n , to the successive stage numbers from 1 to 11 (and thus to the number of segments present at the time); $(TS_1 - TS_0)$, to the initial length of the first segment at the end of stage 1; and Δs , to the increase of segmental length per stage (0.0097 mm.). The expression would mean that the first segment grows in length by a constant amount Δs during each stage.

Since every other thoracic segment is known to start off with an identical value for $(TS_1 - TS_0)$, viz., 0.03 mm., it could be possible that other thoracic segments also increase a constant amount Δs during each stage. If that were true, then the newest segment, at any given stage, would have a length of $(TS_1 - TS_0)$, the segment immediately preceding it a length of $(TS_1 - TS_0) + \Delta s$, the third but last a length of $(TS_1 - TS_0) + 2\Delta s$, . . . etc., and the first segment again a length of $[(TS_1 - TS_0) + (n - 1) \cdot \Delta s]$. In other words the length of the entire thorax, being the sum of individual segments, should be the sum of an arithmetical series whose first term is $(TS_1 - TS_0)$ and whose last term is $[(TS_1 - TS_0) + (n - 1) \cdot \Delta s]$. This can be put as

$$T_n = n \cdot (TS_1 - TS_0) + \frac{n(n - 1)}{2} \cdot \Delta s \quad (2)$$

where T_n is the total thoracic length at stage n ; and $(TS_1 - TS_0)$, the constant length of the newest segment (or the length of the first segment when in stage c). Taking Δs as 0.0097 mm. and $(TS_1 - TS_0)$ as 0.03 mm., T_n for successive values of n can be calculated. These calculated values are compared with the observed values for thoracic length in Table II; the largest discrepancy is only approximately 5 per cent, and the original suggestion is thus shown to be fact, i.e., every thoracic segment grows in length for a constant amount Δs , in each stage of the thoracic period.

A similar approach can be employed to analyze thoracic changes in width. While in stage 0 thoracic length T_n is also 0, the anterior width of the presumptive thorax is already 0.142 mm. (C). In stage 1, the width of the first segment is 0.144 mm. (Table I), and the initial increase $(W_1 - C)$, analogous to $(TS_1 - TS_0)$ in equations 1 and 2, is therefore 0.002 mm.; the anterior width of the presumptive second segment is again $C = 0.142$ mm. (each segment after stage c being regarded as a short cylinder). In succeeding stages, the width of the first segment increases 0.002, 0.004, 0.005 mm. . . . etc. (Table I). The increments per stage are then not constant, as they were for segmental length, but the figures suggest that the increases of the increments per stage might be constant. If the increment in stage 2 were 0.003 instead of 0.002 mm., the increase Δw over the initial increment $(W_1 - C)$ would be 0.001, and $(W_1 - C) + \Delta w$ would represent the increase in width during stage 2. Similarly $(W_1 - C) + 2\Delta w$ and $(W_1 - C) + 3\Delta w$ would indicate the increases during stages 3 and 4 respectively. In general,

$$(W_n - W_{n-1}) = (W_1 - C) + (n - 1) \cdot \Delta w \quad (3)$$

would be true, where $(W_n - W_{n-1})$ represents the increase in width of the first segment during stage n . The total width increase of the first segment during the first n stages would then be the sum of an arithmetical series whose first term is $(W_1 - C)$ and whose last term is $[(W_1 - C) + (n - 1) \cdot \Delta w]$, for similar reasons as in thoracic length; or

$$W_n - C = n \cdot (W_1 - C) + \frac{n(n-1)}{2} \cdot \Delta w \quad (4)$$

and

$$W_n = C + n \cdot (W_1 - C) + \frac{n(n-1)}{2} \cdot \Delta w \quad (5)$$

TABLE II

Calculated and observed magnitudes of certain larval body regions, in millimeters

Stage No.	Thoracic length		Width of 1st thoracic segment			
	Observed	Calculated	Observed	Calculated		
1	0.030	0.030	0.144	0.144		
2	0.070	0.069	0.146	0.147		
3	0.122	0.119	0.150	0.151		
4	0.185	0.178	0.155	0.156		
5	0.242	0.247	0.162	0.162		
6	0.311	0.325	0.167	0.169		
7	0.390	0.413	0.179	0.177		
8	0.480	0.511	0.191	0.186		
9	0.600	0.619	0.209	0.196		
10	0.733	0.736	0.225	0.207		
11	0.861	0.863	0.245	0.220		
	Length of post-abdomen		Length of a post-abdominal segment		Width of 12th segment	
	Observed	Calculated	Observed	Calculated	Observed	Calculated
12	(0.29)	(0.28)	(0.041)	(0.04)	0.150	0.147
13	(0.325)	(0.30)	(0.054)	(0.05)	0.160	0.154
14	0.38	0.36	0.06	0.06	0.170	0.163
15	0.45	0.45	0.08	0.075	0.185	0.174
16	0.58	0.57	0.09	0.095	0.190	0.187
17	0.73	0.72	0.12	0.12	0.200	0.202
18	0.91	0.90	0.15	0.15	0.210	0.219
19	1.13	1.11	0.18	0.185	0.230	0.238

Taking for $(W_1 - C)$ and Δw the values 0.002 and 0.001 mm. respectively, W_n has been calculated for successive values of n , and the comparison with the observed values is shown in Table II. The percentage discrepancies are greater than those observed for thoracic length, but nevertheless insignificant in view of the greater difficulty of taking accurate measurements of entities of so much smaller magnitude. It is to be concluded that the width of the first thoracic segment grows similarly as the length of the thorax, i.e., by adding, in each stage, another term

of an arithmetical series in which consecutive terms differ by a constant amount Δw .

It must now be shown that other thoracic segments also increase in width according to equations 4 and 5; actual measurements for these segments have not been taken, but the proof can be arrived at indirectly. It is known from observation that the lateral thoracic contours are straight lines converging posteriorly. The angle of taper α (Fig. 2) is always expressed by

$$\tan \alpha = \frac{W_n - C}{2T_n} \quad (6)$$

and this angle, on calculation, is seen to be very nearly constant for successive values of n . For $n = 1$ and $n = 11$, $\tan \alpha$ equals 0.033 and 0.045 respectively; the average from all eleven values is 0.039, corresponding to an angle of $2^\circ 18'$, $\pm 15'$. Since the contours are then straight lines, with a constant taper in all thoracic stages, the taper of individual segments must also be constant and identical, i.e., $(W_n - W_{n-1})/2TS_n$; as the length TS_n of a given segment in a given stage can be shown to be equal to the length, in the preceding stage, of the segment immediately anterior to it, an analogous equality must obtain for the width of a segment, for the taper in each case must be identical. In other words, when the width of the first segment is W_n , the width of the succeeding segment is W_{n-1} , in the same stage; this proves however, by extension, that all thoracic segments must increase in a manner identical to the first, since W_n and W_{n-1} represent sums of the same arithmetical series as that in equation 5, W_n containing one term more than W_{n-1} .

The segmental abdomen during the thoracic phase maintains a constant length ($A = 0.249$ mm.) and a constant anterior width ($C = 0.142$ mm.). The posterior width WU , identical to the "width of the urosome," however increases (Table I). The angle of taper β , therefore, expressed by

$$\tan \beta = \frac{C - WU_n}{2A}, \quad (7)$$

decreases. Stated in other words, the convergence of the abdominal contour-lines gradually diminishes. A stage will eventually be reached at which the thoracic and abdominal contours will form continuous straight lines, the thoracic contours having a constant taper (equation 6); at this time

$$\tan \alpha = \tan \beta$$

and

$$\frac{W_n - C}{2T_n} = \frac{C - WU_n}{2A} \quad (8)$$

from which WU_n can be calculated, all other terms being known. WU_n from equation (8) is 0.123 mm.; the value of WU_n closest to this in Table I is 0.125 mm. in stage 11. It follows therefore that the thoracic and abdominal contours become continuous straight lines as the end of the thoracic period of development is reached.

For analytical purposes thoracic shape during the thoracic period can be regarded as a regular cone from which the tip was cut off (frustrum of a cone). Dorso-ventral extent at any level would be very nearly equal to the lateral width

at that level. The diameters of the end faces of the frustrum can thus be assumed to be W_n and W_1 respectively, and since the length of the frustrum is always given by T_n , the volume and the surface area of the thorax can be approximated by the use of known geometrical formulae. If the volume V_1 of the first segment is known the total thoracic volume V_n at any stage can also be calculated from a sum-of-a-series equation, of the general form

$$V_n = n \cdot V_1 + \frac{n(n-1)}{2} \cdot \Delta v, \quad (9)$$

which must obtain, since both length and width changes are governed by such equations. Furthermore, Δs and Δv are obviously related mathematically to Δv . In sum, if the initial size and shape of the thorax ($n=1$), and the values Δs and Δv are known, the size and shape of the thorax at any further thoracic stage can be predicted.

The abdominal phase of development

Abdominal growth.—At the beginning of the abdominal period, the segmental abdomen starts to grow in length, having been constant before. During stages 12 and 13 the abdominal increases are 0.08 mm. per stage, or almost exactly $8 \times \Delta s$ (Table I); since the initial abdominal length at the beginning of stage 12 (or at the end of stage 11) is 0.249 mm. or approximately 8×0.03 m., it follows that during the genital period each 0.03 mm. portion of the segmental abdomen grows an amount Δs per stage. In other words, the segmental abdomen behaves as though it were already cut up into its eight segments, and each of these hypothetical segments has the same antero-posterior growth potential as thoracic segments when first laid down, viz., increasing Δs per stage after having a length of 0.03 mm. If the 12th segment were laid down in the manner in which thoracic segments are formed, it would reach stage c at a length of 0.03 mm. But after stage 11 the entire segmental abdomen has already started to grow, at a rate of Δs per stage per 0.03 mm. Thus at the end of stage 12 when the 12th segment reaches stage c , it will be $0.03 + \Delta s$, or 0.04 mm. instead of 0.03 mm. long; the entire segmental abdomen should then be eight times 0.04, or 0.32 mm., and the post-abdomen 0.28 mm. long. Analogously during stage 13, each 0.04 mm. portion of the segmental abdomen will now add an amount Δs , so that segment 13 when in stage c will be 0.05 mm. and the entire segmental abdomen eight times 0.05, or 0.40 mm. long. At this point the genital region should be 0.10 (2×0.05) mm. and the post-abdomen 0.30 mm. long. Actual figures in Table I support such an interpretation rather well, and the conclusion is justified that during the genital period the segmental tissue of the abdomen acquires the same growth potential in length as that of equivalent amounts of thoracic tissue during the thoracic period.

The genital region continues to grow in length at the indicated rate, as the data in Table I tend to show. The post-abdomen would similarly do so, were it not for the fact that another change in the mode of growth occurred at the end of stage 13. Successive post-abdominal lengths P_m from stage 13 on are 0.325, 0.38, 0.45, 0.58 mm. etc., in other words the increments are increasing. A sum-of-a-series expression, similar to that for thoracic width changes, fits these figures very

closely, i.e.,

$$P_m - P_0 = (P_1 - P_0) \cdot m + \frac{m(m-1)}{2} \cdot \Delta p \quad (10)$$

and

$$P_m = P_0 + (P_1 - P_0) \cdot m + \frac{m(m-1)}{2} \cdot \Delta p \quad (11)$$

where P_m represents the total post-abdominal length for stages 14 to 19; P_0 , the initial length at the end of stage 13; P_1 , the length at the end of stage 14; m , the successive integers from 1 to 6; and Δp , the increments per stage over the initial increase ($P_1 - P_0$). The theoretical value for P_0 was previously seen to be 0.30 mm., and with 0.06 and 0.03 for ($P_1 - P_0$) and Δp respectively, the calculated values for P_m compare well with the observed ones (Table II).

If equation (11) is divided by six, the growth formula for individual segments is obtained, since each of these segments is one-sixth of the entire post-abdomen;

$$PS_m = PS_0 + (PS_1 - PS_0) \cdot m + \frac{m(m-1)}{2} \cdot \Delta(p) \quad (12)$$

PS_0 , PS_1 , and $\Delta(p)$ are 0.05, 0.06 and 0.005 mm. respectively, and ($PS_1 - PS_0$) is therefore 0.01, or very closely Δs ; thus the initial increase of the presumptive segments 14 to 19, at the beginning of the post-abdominal period, is identical to the increase of these tissues during stages 12 and 13, and this increment is then augmented by a constant amount $\Delta(p)$ in each subsequent stage. What is responsible for this change in the mode of growth of post-abdominal segments? It is more than likely that non-formation of appendages is related to this, inasmuch as newly formed tissue will not be diverted for the establishment and subsequent growth of appendage buds; augmented growth of the segments would therefore be facilitated. It can now be stated in general, that while body segments are formed, length increments per stage for all segments are constant, but the increments may be added to an initial length as in thoracic and genital segments, or to an initial increase of length, as in post-abdominal segments.

As in thoracic segmentation, the anterior width of the segmental abdomen has the constant value $C = 0.142$ mm., during the abdominal period. This value is the anterior abdominal width at the end of stage 11, and the anterior width of the presumptive 13th segment at the end of stage 12. The 12th segment, by this time, has attained a width of 0.15 mm. (Table I), and in succeeding stages this width increases to 0.16, 0.17, 0.185 mm. . . . etc. As for thoracic width the increases are found not to be uniformly constant, and a sum-of-a-series expression again approaches the data best, i.e.,

$$WA_r - C = r \cdot (WA_1 - C) + \frac{r(r-1)}{2} \cdot \Delta wa \quad (13)$$

and

$$WA_r = C + r(WA_1 - C) + \frac{r(r-1)}{2} \cdot \Delta wa \quad (14)$$

where WA_r represents the width of the 12th segment at a stage r of the abdominal period; ($WA_1 - C$), the initial increase in width during stage 12; Δwa , the increase in width, per stage, over the increment during the preceding stage; and r ,

the successive integers from 1 to 8. If for $(WA_1 - C)$ and Δwa 0.005 and 0.002 mm. respectively are taken, the calculated values for WA_r compare well with the observed ones (Table II).

Other abdominal segments can be shown to follow a similar mode of growth in width. The lateral contours being straight lines, the angle of taper β is expressed by

$$\tan \beta = \frac{WA_r - WU_r}{2A_r} \quad (15)$$

where A_r is the length of the entire segmental abdomen, i.e., genital plus post-abdominal lengths, and other values as before. $\tan \beta$, when calculated from Table I for successive values of r , centers about the average of 0.036 ± 0.004 ; in other words, the abdominal taper does not only remain constant during the abdominal period, but this taper is also practically identical with that reached by the segmental abdomen at the end of stage 11 (cf. above, equation 8).

Unlike thoracic segments, which start development at stage c with the same length as that of more anterior segments at stage c , the abdominal segments begin development at a length identical with that of more anterior segments at the same time. In maintaining a constant taper, the initial increase of any presumptive thoracic segment over the width C is always expressed by the first term of the series applying to thoracic width (equations 3, 4, and 5), and the later a segment arises the fewer terms of the series can it add to its width during the thoracic period. Since abdominal segments have now also been shown to maintain a constant taper, and since their lengths at stage c are equal to those of more anterior segments already beyond stage c , an analogous relation must similarly exist for segmental width; namely, the initial increase of a presumptive abdominal segment over the width C must be identical to the width increase experienced by other abdominal segments at the same time. If $(WA_1 - C)$ in equation (13) represents the initial increase of segment 12, then $(WA_2 - WA_1)$ would do similarly for segment 13. In other words, the width of both segments follow the same series, but the second term for segment 12 becomes the first term for segment 13; the third term for segment 12, similarly, becomes the first term for segment 14, etc., and the eighth and last term for segment 12 is the first and last term for segment 19. Thus as with thoracic segments, the later an abdominal segment arises the fewer terms are added to its width, but while the width increases of thoracic segments start with the same and end with consecutive terms, those of abdominal segments start with consecutive and end with the same terms of the series.

It should be observed parenthetically that equation (13) may have a slightly different constant Δwa for the genital and post-abdominal segments respectively, reflecting the different modes of growth in length of these two groups of segments; or, if the constant is actually identical the lateral abdominal contour would theoretically not be an exact continuous straight line, but rather two straight lines with slightly different taper, joined between segments 13 and 14. In either case, the difference would be so small as to be unnoticeable in practice; with the present techniques of observation and measurement, a single series relation holds for both groups of segments, and even if two separate series could be established with finer means, the principle of growth in width as outlined above would nevertheless hold.

As for segmental growth in length, a general conclusion can now be stated for

growth in width, viz., width increments per stage for all body segments are constant, and the increments are always added to an initial increase in width. The combined generalization is also true, that total segmental mass increments per stage are constant, and the increments are added either to initial masses or to initial increases of mass.

Thoracic growth.—One of four possible reasons could *a priori* be advanced in an attempt to account for the differential size changes in the thorax, such that the 5th segment ultimately becomes largest, during the abdominal period; i.e., either the segment rudiments in stage *a* differ in initial size but follow the same growth curves; or the analogous converse; or either the rudiments have both equal initial size and identical growth curves; or the analogous negative. Since for all thoracic segments four stage-intervals elapse between stage *a* and stage *c*, length and width magnitudes at stage *c* are identical, and the increments per stage, no matter at which segmental stage, are identical (i.e., Δs), only the conclusion is admissible that thoracic segment rudiments have equal initial sizes and follow growth curves of the same shape. Under such conditions there are two factors which must be held responsible for the observed growth of thoracic segments, i.e., the time lag in the formation of consecutive segments, and segmental age. The time lag fully accounts for the regular gradation of segmental size at the end of the thoracic period and for the constant taper of the thorax; as will be demonstrated below, the influence of this original time lag carries over importantly into the abdominal phase, and this, together with the factor of segmental age, can indeed be made the basis for a consistent interpretation of the manner of thoracic growth.

Data in Table I show that thoracic length remains constant during the genital phase. Hereafter the values for length fit the equation

$$T_m = T_{m-1} + (T_1 - T_0)_m - \frac{m(m-1)}{2} \Delta s \quad (16)$$

where T_0 and T_1 represent thoracic length at the end of stage 11 and stage 14 respectively, and m , as before, the integers from 1 to 6. With 0.86 and 0.92 mm. for T_0 and T_1 , and Δs as before, successive calculated values for T_m are 0.92, 1.03, 1.18, 1.36, 1.56, and 1.77 mm., significantly close to the observed data; the increases per stage are therefore 0.06, 0.11, 0.15, 0.18, 0.20, and 0.21 mm., and the differences between the increases are seen to diminish in a regular manner.

The scheme in Table III will account for such a series of increases. The figures in this table represent multiples of Δs and they show the length increase of the indicated segment during the indicated stage. Sums of figures in vertical rows, multiplied by Δs , indicate the increases of the entire thorax during the given stages, and successive sums are seen to be equal to the values for the increases per stage as calculated from equation (16). Horizontal sums, multiplied by Δs , give the total increments of any thoracic segment during the post-abdominal period. This scheme is reproduced somewhat differently in Table IV, in which the figures, multiplied by Δs , indicate directly the size of any of the 19 body segments at any of the 19 developmental stages; vertical sums have meanings analogous to equivalent sums in Table III.

It will be observed that all formulae previously deduced in connection with length increases are inherent in the figures in Table IV; observational data are

also incorporated. For example, the first segment when reaching maturity in stage 7 has a length of 0.09 mm. (cf. Table I). Succeeding thoracic segments must also mature at this size, in consequence to the equality of their growth curves; thus segment 5 is shown to mature in stage 11 when the 19th segment appears in stage *a*, and segment 11 in stage 19, in conformity to the observational data in Table I. The scheme in Table IV also shows well the successive segmental proportions in the thorax during the abdominal phase. In stage 16, segments 1 and 2 are longest, in stage 17 similarly segments 2 and 3, etc.; maximal segmental length thus shifts caudad, fully corroborating observation.

Segmental growth of the thorax as indicated in the table can be interpreted provided two assumptions are postulated, i.e., (*a*) a segment can no longer grow by regularly increasing amounts after having passed through 14 segmental stages.

TABLE III

Scheme of segmental increments, in multiples of Δs , in the thorax during the post-abdominal phase of development

	Stage						Total segmental increases
	14	15	16	17	18	19	
Segment							
1	0	1	1	1	1	1	5
2	0	1	2	2	2	2	9
3	0	1	2	3	3	3	12
4	0	1	2	3	4	4	14
5	0	1	2	3	4	5	15
6	1	1	1	1	1	1	6
7	1	1	1	1	1	1	6
8	1	1	1	1	1	1	6
9	1	1	1	1	1	1	6
10	1	1	1	1	1	1	6
11	1	1	1	1	1	1	6
Total thoracic increases	6	11	15	18	20	21	

counted from stage *c*, and (*b*) a segment, in order to grow by increasing amounts at all, must have matured within the first 6 segmental stages of its existence, counted from stage *c*. These two provisions constitute the limiting conditions of segmental age.

Table IV reveals that only the first 5 segments fulfill the second condition; segments 6 to 11 would also have matured in 6 stages of their individual existence, were it not for the fact that no thoracic growth takes place during the genital period, and maturation of the posterior thoracic segments is therefore delayed by two stages. Thus only the first five segments would be able to grow by increasing amounts, whenever such growth was made possible. It has been shown previously that at the beginning of the post-abdominal phase, the post-abdomen ceases to grow by constant increments and begins growth by increasing increments, with an initial

increase during stage 14 equal to that of stage 13. Apparently the phenomenon of increasing increments at this time is not confined to the post-abdomen but also affects thoracic segments, subject to the limiting provisions stated above. Thus the first five segments have an initial increase equal to the increment during stage 13, viz., 0; segments 6 to 11, not fulfilling condition (*b*), simply continue at their former constant rates, viz., Δs per stage (cf. data in Table III, under increases during stage 14). From here on, the first five segments augment their increases by Δs in every stage, until their 14th segmental stage is passed; then, by assumption, the increment of the 14th segmental stage can no longer be augmented, but

TABLE IV

*Scheme of growth of body segments, in multiples of Δs
(a refers to segmental stage a of any given segment)*

	Stage																		
	Thoracic phase											Genital phase		Post-abdominal phase					
Seg- ment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	3	4	5	6	7	8	9	10	11	12	13			13	14	15	16	17	18
2		3	4	5	6	7	8	9	10	11	12			12	13	15	17	19	21
3			3	4	5	6	7	8	9	10	11			11	12	14	17	20	23
4				3	4	5	6	7	8	9	10			10	11	13	16	20	24
5	a				3	4	5	6	7	8	9			9	10	12	15	19	24
6		a				3	4	5	6	7	8			9	10	11	12	13	14
7			a				3	4	5	6	7			8	9	10	11	12	13
8				a				3	4	5	6			7	8	9	10	11	12
9					a				3	4	5			6	7	8	9	10	11
10						a				3	4			5	6	7	8	9	10
11							a				3			4	5	6	7	8	9
12							(a)					4	5	6	7	8	9	10	11
13								a					5	6	7	8	9	10	11
14								(a)						6	7.5	9.5	12	15	18.5
15									a						7.5	9.5	12	15	18.5
16									(a)							9.5	12	15	18.5
17										a							12	15	18.5
18										(a)								15	18.5
19											a								18.5

is retained as a constant increment till growth stops altogether. Thus segment one has increased its increment of zero by Δs at the end of stage 15 (Tables III and IV); but at this point its 14th segmental stage has already been passed and hereafter only a constant increment of Δs per stage is possible. Segment 2 on the other hand is younger than segment one, being laid down in stage *c* with a time lag of one developmental stage. By the end of stage 15, therefore, when segment one has just passed its 14th segmental stage, segment 2 has only passed its 13th segmental stage and its increment of Δs during stage 15 can be augmented once more by Δs ; when the 2nd segment has passed its 14th segmental stage, its in-

creases in subsequent stages will therefore be $2 \Delta s$ per stage. Similarly, segments 3, 4, and 5, each being one stage younger than the preceding segment, are able to augment their increments by Δs 3, 4, and 5 times respectively, before they complete the 14th segmental stage. Segment 5 in consequence is as long as segment 4 at the end of stage 19, but the former will continue to grow at a rate of $5 \Delta s$ per stage, while the rate of the latter can only be $4 \Delta s$ per stage; at any time after stage 19 therefore the fifth segment will be longest.

Analogous changes occur with regard to thoracic growth in width, and the thoracic cone-frustrum of stage 11 gradually assumes the shape of a barrel, with the "waist" at segment 5 after stage 19. The dorsal thoracic curvature of the animal, arising similarly after stage 11, can also be interpreted as a result of differential segmental increases in a dorsal direction, according to a scheme resembling that in Table III.

The genital segments have been noted to mature, i.e., to form a broodpouch, in stage 18. Table IV reveals that at the end of this stage, segment 12 has just completed its 6th, and segment 13, its 5th stage of segmental development, counted from stage *c*. Thus both segments fulfill one of the two age conditions assumed for thoracic segments: the fulfillment of the other might be expected. Observation proves that this is actually so. Genital segments of older larvae are known to bulge considerably beyond the general abdominal contour, giving them a knobby appearance. This could not be possible if the constant increases observed up to stage 18 were maintained any further; rather, after stage 18 the initial increment of an increasing rate will again be equal to the increase during the stage just passed, viz., Δs , and during a 20th stage this increment will be augmented by a given amount, during a 21st stage by twice this amount, etc., till the 14th segmental stage is passed.

The post-abdominal segments have previously been shown to grow by regularly augmented increases as soon as they are laid down. But since these segments bear no appendages, stage *c* for them is equivalent to attainment of maturity, as already observed above. Maturity thus proves to be an important temporal threshold for all body segments, and the statement that augmented growth will occur in any mature segment, provided maturity was reached in a definite time, has general application; the concept of segmental maturity is apparently not only a working hypothesis, as has been assumed at the start, but seems to have real biological meaning.

There is no doubt that the scheme of growth here presented describes correctly the actual events of later thoracic development; but the assumptions, while justified by the interpretations they allow, still remain to be explained. Only tissue culture studies will be able to reveal why segments not matured in the first 6 stages of existence are at too early a stage of development, and why segments after 14 stages of existence are at too advanced a stage to do more than keep up a constant rate.

Growth of appendages; integration of segmental development

In the preceding section it has been reasoned that segment rudiments in the thorax have equal initial size and identical growth curves; observation tends to confirm not only this but also that equal-sized rudiments develop for all body segments. It can be assumed that in these rudiments certain tissue masses (ap-

pendage rudiments), initially also of equal size and of equal growth capacity in equal times, differentiate independently towards the establishment of appendage buds. Such buds however never appear in the post-abdomen, and when they appear in other regions they may develop into swimming appendages or into a broodpouch. In the evidence presented in Tables I and IV, an important clue can be found to at least one of the factors preventing serial analogy despite the observed serial homology in appendageal development.

Every thoracic appendage rudiment reaches the bud stage after an interval of four developmental stages. The segment as a whole is at stage c at this point, and the appendage buds of any thoracic segment must be of identical size, due to the identity of initial size and of growth capacity for all appendage rudiments, and of identical shape, since every thoracic segment at stage c has identical proportions. Enough appendageal tissue has apparently been manufactured, during the four preceding stages, to initiate the development of a swimming appendage.

When a genital segment reaches stage c , 4 + and 5 developmental stages have elapsed since stage a . The appendage rudiments therefore have time to manufacture proportionately more appendageal tissue, at the same intensity as that of thoracic rudiments. If the genital segments in stage c had larger sizes, proportionate to the longer time interval available, the appendage buds of genital segments would have the same size and shape as those of thoracic segments. However, both the length and the width of genital segments are greater in stage c than the size which would be proportionate to the longer time of formation. The length of any thoracic segment when laid down is 0.03 mm., for example, and four developmental stages have elapsed since stage a ; the length/time ratio is thus 0.03/4. In genital segments this ratio is larger, viz., 0.04/4 + and 0.05/5, and analogously for width. Appendageal tissue in genital segments can therefore not be developed in sufficient quantity, in proportion to segmental size, to produce appendage buds of dimensions equal to those of thoracic buds, even though more time is available. Genital buds will thus be relatively smaller and flatter, and the amount of appendageal tissue manufactured will be spread more thinly over the presumptive appendage region; the quantity of tissue present per unit area is apparently already below the threshold necessary for the formation of comparatively specialized swimming appendages, and only enough tissue is available to initiate the formation of a relatively simple sac.

In post-abdominal segments at stage c the size/time ratio becomes progressively larger still, and appendageal tissue consequently cannot even accumulate in quantities sufficient to form a bud.

After the appendage buds are laid down, an appendage retains a definite size-proportionality to the segment bearing it. When, for example, the thoracic contour is a straight line, during the thoracic phase of development, the line joining the tips of the appendages on one side is also a straight line, and, as with the segments themselves, the time lag in bud formation accounts for the taper. Similarly, as the thorax gradually becomes barrel-shaped in the abdominal phase, the transformation is reflected in differential length increases in the appendages, and when the appendageal tips on one side of the body are joined by a line, the result is an analogously barrel-shaped contour.

From the above analyses, the following integrated sequence of events becomes apparent with regard to segmental development.

Shortly before hatching segmental rudiments of equal size begin to be formed, at a rate of one per developmental stage; with a time lag of four stages, segments are constricted off in posterior succession, all with constant initial sizes and increasing by a constant amount during each stage. As the first segment reaches maturity, the rate of segment rudiment formation increases to two per stage. Rudiments are laid down at this rate till the newest rudiment appears at the posterior end of the segmental abdomen which latter had so far maintained a constant length. The last formed rudiment happens to be the 19th and by this time, 11 segments have been constricted off, five of which have already matured.

The process of rudiment deposition and segment constriction could be assumed to go on at length, were it not for the fact that the "end" of the animal has been reached. This is apparently the cue for a general change in the mode of growth. The entire segmental abdomen begins growth, increasing as yet equal amounts per stage, and the thorax ceases to grow. After two genital segments of equal size are formed another general change occurs, to the effect that hereafter any segment maturing within a definite time may grow by augmented increases, as described in detail above. This type of growth is maintained, in each segment in which it takes place until the 14th segmental stage is passed, whereupon the total increment of the 14th stage is reproduced without further increase in each succeeding stage. Segments not matured within the required time continue to grow by constant increments. The eventual result of this varied manner of growth, maintained up to sexual maturity, is the barrel-shape of the thorax, the presence of a dorsal thoracic curvature, the knobby appearance of the broodpouch segments, etc.

DISCUSSION

Throughout the present analysis of metamerism in *Artemia salina*, the time scale employed was that of developmental stages, defined as the number of body segments present. It must be eminently realized that this is a scale of relative, biological time. Events in nature take place in a space-time continuum, and to *Artemia* equivalent happenings in space, i.e., the establishment of segments, must be correlated to the passage of equivalent units of (relative) time, i.e., what here had been called "stages." In hours and minutes, segment formation occurs of course not in equivalent times, since the phenomenon is dependent on the environment on the one hand, and on changes in growth rates with age on the other. *Artemia* and other similarly primitive forms are particularly suited for a ready identification of relative time, but in segmented animals of greater complexity, as well as in non-segmented groups, "equivalent happenings in space" cannot be picked out with comparative ease, and it will be more difficult to tell what the relative time scale actually is; but that it is intrinsically present in biological phenomena has already been acknowledged by others. Thus Needham (1942), after briefly reviewing the pertinent literature, states:

"Mouse time must bear the same, or a similar, relation to elephant time as mouse spatial magnitudes to elephant spatial magnitudes. Indeed, unless the time factor is brought into account, we may understand morphological similarity, but we can never hope to understand physiological, still less embryological, similarity."

Measurements on *Artemia* in absolute time would never have brought to light the truly amazing simplicity of the laws of segment formation, as given by the series and the sum-of-series formulae, and in terms of relative time these formulae assume a simple biological meaning, viz., (a) that equivalent spatial events take place during equivalent relative times, and also (b) that equivalent spatial events take place in tissues of equivalent relative age. For illustration, the thorax during the thoracic period of development may be considered, where the increments per stage of $(TS_1 - TS_0)$ and $(W_1 - C)$ (equations 1 and 3) are indeed equivalent and constant, and where every other segment grows similarly in this same manner; summation of the increments must then result in the sum-of-series expression. Analogous interpretations, based on the idea of spatial and temporal equivalence can be adduced in every other case in which the formulae hold, i.e., virtually for the entire period of segmental development. Before and after this period, relative time is of course still operative, but its expression is latent, inasmuch as its passage is not paralleled by morphological events clearly identified as equivalent. The same would be true for the majority of living organisms, but it can be asserted with a fair amount of logical conviction, that if and when it will be possible to make explicit the relative time scales of living organisms as a whole, size increments in relative time units will be found to be equivalent, and series formulae of linear, quadratic, and perhaps even of higher degree will be found to hold.

It should in general be useful to have a specific term to distinguish relative biological time from absolute duration; the concept as a whole might be called "biochronism," and the relative time scale could be said to have one "biochron" as its unit. Also, in order to transcend the usual connotations of "growth rate," "biochronal rate" could be substituted. Whenever in the text above "increase per developmental stage" was mentioned, "increase per biochron" was really implied. In this connection, the type of analysis in the present report is clearly different from "allometric," "heterauxetic," or "heterogonic" inquiries. The term "morphometry" is suggested to indicate generally any quantitative appreciation of organic size, shape, and time as an integrated dynamic pattern. While it is realized that apologies are in order, more or less categorically, for the introduction of any new term into present-day biology, it should be kept in mind that new terms become unavoidable as different methods of inquiry and fresh fields of study appear.

Two immediate issues have not been touched on at all in the present analysis. First, what determines the changes in the mode of segmental growth at the end of both the thoracic and the genital periods? That the changes occur is fairly definitely established, and this would support the view that division of segmental development into periods is real, i.e., physiological as well as morphological. But beyond that, speculation into the nature and history of the changes would be futile, for lack of direct evidence. Secondly, and this is the fundamental question in the study of metamerism, why are segments formed at all? It will readily be admitted that even an attempt to answer this problem can only be made after a great deal more is known about segmentation phenomena as a whole.

Excepting these two questions however, the final size and shape of *Artemia* nevertheless has here been accounted for in terms of initial body proportions much as Berrill (1941) has done for the ascidian, *Botryllus*. When copepods, crayfish, and other diverse crustacean forms of higher evolutionary rank are considered, similarly in possession of a barrel-shaped thorax and a straight tapering abdomen,

it is perhaps justifiable to reflect that Crustacea as a group might have evolved with a single and basic geometrical pattern of growth.

SUMMARY

1. Growth and the dynamic pattern of segment formation in excysted larvae of *Artemia salina* have been quantitatively studied. The final shape of *Artemia* at sexual maturity can be accounted for in terms of initial shape at hatching.

2. In analyzing the pattern of metamerism, the stages of development are gauged by the number of body segments present. Growth during the entire period of segment formation is found to be governed by arithmetical series and sum-of-series relations, implying that growth increments per stage over either initial sizes or initial increases are constant and identical for thoracic, genital, and abdominal segments, respectively. Later transformations of larval shape, resulting in the barrel-shape of the thorax, the presence of a dorsal thoracic curvature, the knobby appearance of the genital segments, and the presence of a straight tapering abdomen, are accounted for analytically on the basis of concepts concerning the age of segments and the time lag involved in segment formation.

3. The presence, absence, and the difference of structure of appendages are shown to be determined, at least in part, by the size of segments when first laid down, and by the time available for appendage rudiments to form appendageal tissues..

4. The time scale employed in the analysis of the segmentation pattern in *Artemia* is interpreted to be a relative, biological one, and the meaning of the series formulae with regard to this relative scale is illustrated. The notion of "bio-chronism" is introduced, as a general concept applying to biological events in relative time.

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